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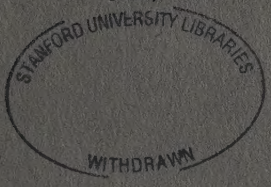
A THESIS

Presented to the Faculty of the Graduate School
of Cornell University for the Degree of

DOCTOR OF PHILOSOPHY

By William J. Robbins

June, 1915



Reprinted from the American Journal of Botany, 5: 234-258
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INFLUENCE OF CERTAIN SALTS AND NUTRIENT SOLUTIONS ON THE SECRETION OF DIASTASE BY PENICILLIUM CAMEMBERTII[1]

WILLIAM J. ROBBINS

Much has been published in recent years concerning the regulatory production of enzymes, but the investigations have been largely confined to the influence of the organic compounds on the production and secretion of enzymes. Little attention has been devoted to the effects of the mineral elements on enzyme formation. It would seem that such studies are of importance for several reasons. They might lead, for example, to a better understanding of the origin of enzymes. They should lead also to a clearer realization of the rôles played by the mineral elements in plant nutrition. In this regard it is significant that there exists a relation between potassium and carbohydrate formation in green plants, and also a relation between calcium and starch translocation. The rôles played by potassium and calcium in these processes are not understood, but it is possible, and it has been suggested by some investigators, that they condition the formation of certain of the carbohydrases.

It is also conceivable that studies of the effect of mineral salts on the secretion of enzymes would be important in the realm of plant pathology, particularly in the problem of disease resistance in plants. The interpretation of the action of distilled water on plants may also be aided by such studies.

As a result of these considerations a problem was evolved comprehending an investigation of the influence of certain single salts, and of certain nutrient solutions with various modifications, on the secretion of diastase by *Penicillium camembertii* Thom. The modifications of the nutrient solutions consisted in the replacement of certain essential radicals of salts by nonessential radicals.[2]

[1] Contribution, Laboratory of Plant Physiology, Cornell University.

[2] It is a pleasure to acknowledge the indebtedness of the author to Dr. Lewis Knudson for suggesting this problem, and for constant aid and assistance in the preparation of the manuscript.

At the outset of the investigation it was found that a considerable amount of experimentation would be necessary in order to obtain adequate methods of research. It was necessary to devise a new method for measuring starch digestion. It was also essential to determine the significance of the number of spores sown in its bearing on the rate of digestion, as well as the effect of various kinds of distilled water on the rate of digestion. Rather extensive data were obtained in the preliminary experiments, of which only the salient facts are presented.

Methods and Materials

Glassware.—The vessels used in these experiments were all of Jena glass. They were first cleaned with soap and water and chromic acid cleaning mixture, rinsed well with tap water and distilled water, and finally rinsed with the water used in the experiments.

Chemicals.—The chemicals were all of high grade, either Baker's analyzed or Merck's highest purity. The starch used was Merck's soluble starch, which is prepared from potato starch according to Lintner's method as described by Allen (1909). A solution of this starch, when the solvent is redistilled water, permits a small amount of growth of *Penicillium camembertii*. This would seem to show that the starch contains traces of mineral nutrients. According to Ford (1904 *A*), such a preparation contains phosphate, and perhaps organic phosphorus, which cannot be completely removed. According to Thomas (1914), also, the phosphorus present in purified samples of starch is in organic combination.

Water.—The laboratory distilled water, which is derived by distillation from an iron boiler and which is stored in a block tin tank, contains a dark brown precipitate when the last few liters in the tank are drawn. This precipitate consists of some form of iron. The water showed by test with Nessler's reagent no ammonia, and by the diphenylamine reaction no nitrates. Redistilled water was prepared by the double distillation of this water from Jena glass flasks containing acid and alkaline potassium permanganate. This method is described by Jones and Mackey (1897). Water treated with carbon black was prepared by adding 90 grams of moist carbon black, G Elf brand, to 4 liters of distilled water, allowing it to stand for three hours with occasional shaking, and then filtering.

On comparing the growth of *Penicillium camembertii* in a solution

of starch made in these three types of water, distinct evidences of toxicity were noted from the laboratory distilled water. The mycelium in this distilled water was knotty in appearance and in small tufts. The mycelium in redistilled water or in distilled water treated with carbon black was fluffy in appearance and in a connected mat. The apparent toxicity of the distilled water, due perhaps to the presence of iron noted above, led to the use of either redistilled water or distilled water treated with carbon black throughout the experiments.

On comparing the digestion of starch by *Penicillium camembertii* in the three types of water, it was found that this was generally the most rapid in redistilled water, and in most cases slowest in distilled water. The presentation of this phase of the subject is reserved for a future paper.

Sufficient carbon black for the entire investigation was purified at one time by washing it during a period of a week with nine changes of distilled water and two of redistilled water. The carbon black when used contained approximately 72 percent of water. The amount used per 4 liters of water was therefore equivalent to about 25 grams of dry material.

Analytical Methods

In the experiments with nutrient cultures the rate of starch digestion was determined by finding the number of days required by the fungus to digest completely the starch in the culture medium. Complete digestion was determined by the Katz (1898) method. This consists in brief of the removal, daily, of a drop of the culture fluid under antiseptic conditions and the determination of the presence of starch by the use of iodine. When the drop removed shows no coloration with iodine, digestion is considered complete.

In the experiments with single salts it was deemed preferable to determine the amount of starch digested at a given interval from the time of inoculation. This procedure is more accurate and less tedious than the Katz method, and has the advantage of permitting the determination of the starch digestion when the fungous mycelia of all the cultures of a given series are of the same age.

Difficulty was experienced, however, in finding a method suitable for determining how much of the starch originally present in a culture solution had been digested by a fungus growing therein. It was con-

sidered impracticable to determine the amount of starch digested by finding the amount of the reducing sugar produced. A part at least of the sugar produced by the action of diastase is used in the metabolism of the fungus. Furthermore, it has not been demonstrated that *Penicillium camembertii* changes to glucose all of the maltose formed by the action of its diastase on starch. An alternative method might be the following: to determine the reducing value of a part of the solution; to hydrolyze the carbohydrates present in the same volume of the solution by boiling with hydrochloric acid, and to determine the reducing value of this portion. The difference between these two determinations should be the value of the nonreducing carbohydrates of the solution expressed in terms of glucose. This method was considered impracticable because of its tediousness and because of the uncertainty of the nature of the reducing sugars formed from starch by *Penicillium camembertii*.

An attempt was therefore made to evolve a different method of determining diastatic action. It is known that starch and a part of the dextrins are insoluble in a concentrated aqueous solution of alcohol. The sugars formed as a result of the action of diastase on starch, and perhaps part of the dextrins, are soluble in such a solution. This fact was consequently employed in the new method of determining diastatic action here described.

As finally used the method is as follows: By means of a pipette, 20 cc. of the medium are added, slowly and with constant shaking, to 70 cc. of 95 percent alcohol, which is acidified with 1 cc. of hydrochloric acid (sp. gr. 1.18–1.19).[3] This is allowed to stand over night, then filtered through a Gooch crucible, dried at 105° C., cooled in a desiccator over anhydrous $CaCl_2$, and weighed. This method gives us directly the amount of starch and dextrins which have not been digested to the point at which they are soluble in 73 percent alcohol.[4]

The applicability of this method to the determination of diastatic action might be questioned. It was considered necessary, therefore, to compare the measurement of diastatic action by this method with the measurement of diastatic action as found by the amount of reducing sugar produced. This comparison was made by determining the influence of the quantity of diastase on starch digestion, and the influence of time on the digestion by diastase.

[3] Weaker solutions of hydrochloric acid than this may be used. See below.

[4] A mixture of 70 cc. of 95 percent alcohol + 21 cc. of nonalcoholic liquid is a solution of about 73 percent alcohol.

Influence of Quantity of Enzyme.—Fifty cubic centimeters of a 2 percent (approximate) soluble starch solution was placed in each of seven 125 cc. Erlenmeyer flasks. The quantities of 0.1 percent Taka diastase solution used and of the water added in order to keep the concentrations of starch the same throughout are indicated in Table I. After approximately 30 minutes digestion at room temperature, 20 cc. were removed from each Erlenmeyer flask and the undigested starch and dextrins were determined by the alcoholic precipitation method given above. The results follow in Table I:

TABLE I

50 Cc. of 2 Per cent Soluble Starch Plus	Undigested Starch (Mg. per 20 Cc.)	Starch Digested (Mg. per 20 Cc.)	Starch Digested per 1 Cc. of Diastase Solution (Mg. per 20 Cc.)
0 cc. diastase + 32 cc. H_2O	208.0	.....	.....
1 cc. " + 31 cc. "	194.9	13.1	13.1
2 cc. " + 30 cc. "	179.3	28.7	14.3
4 cc. " + 28 cc. "	153.0	55.0	13.7
8 cc. " + 24 cc. "	114.8	93.2	11.6
16 cc. " + 16 cc. "	101.7	106.3	6.6
32 cc. " + 0 cc. "	47.8	160.2	5.0

From these data it would appear that the proportionality between the amount of Taka diastase present and the amount of soluble starch digested, as measured by this method, holds to the point where approximately 25 percent of the original starch has been transformed into substances soluble in 73 percent acid alcohol. This is in fair agreement, considering the fact that the starch and enzyme preparations employed were not purified, with the results obtained by Kjeldahl (1879), Henri (1903), and Ford (1904 *B*), who, working with different starch and enzyme preparations and under different temperature conditions, used the reducing power of the solution as a measure of diastatic action.

The results of the determinations summarized in Table I are presented in the form of a curve in figure 1. On the abscissæ the amounts of Taka diastase solution used are given, and on the ordinates the amounts of digestion. The line parallel to the base represents the starch content per 20 cc. of the original solution.

Influence of Time.—The Taka diastase used contained, according to digestion determinations, insufficient maltase to make certain

the complete transformation of the maltose to glucose. With malt diastase,[5] however, it was found that 0.1 g. of diastase produced no evident hydrolysis of 0.3 g. of maltose in 7 hours at 24° C. With malt diastase as the hydrolyzing agent, therefore, a direct comparison could be made between diastatic action as measured by the maltose produced and diastatic action as measured by the material precipitated in 73 percent acid alcohol.

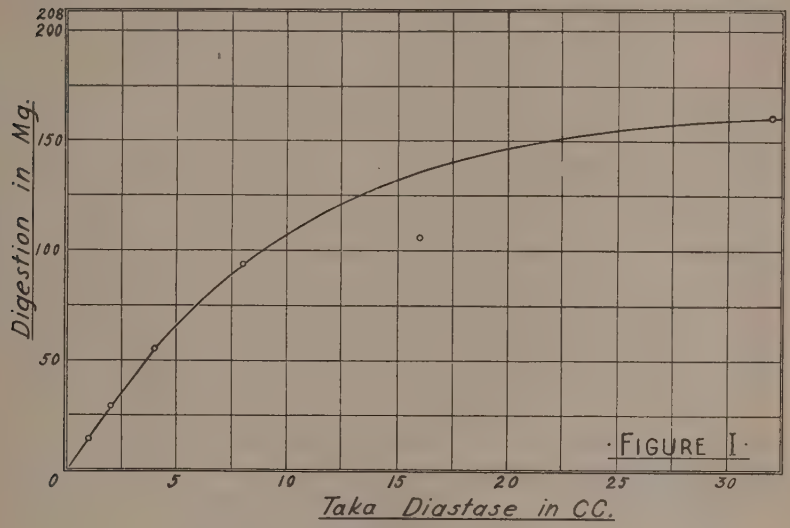

FIG. 1.

About 0.175 g. of malt diastase (Merck's medicinal) was dissolved in 20 cc. of water, and after being filtered to remove all undissolved particles this solution was added to 1,750 cc. of a 2 percent (approximate) starch solution. The mixture was kept in a constant temperature oven at 24° C., but was removed from the oven and replaced each time a determination was made. The data obtained are summarized in Table II:

It is evident that the digestion measured by alcoholic precipitation is greater than that measured by the maltose produced. In other words, a certain fraction of the digestion products is not maltose and

[5] Merck's medicinal.

TABLE II

Time in Min.	Undigested Starch (Mg. per 20 Cc.)	Starch Digested (Mg. per 20 Cc.)	Starch Digested per Minute	Cuprous Oxide (Mg. per 20 Cc.)	Starch Digested Calculated from Maltose Determined (Mg. per 20 Cc.)	Starch Digested per Minute
0	344.1	.....	...	12.4	.....	...
16	334.5	9.6	.60	23.5	6.4	.4
29	325.4	18.7	.64	32.7	13.3	.46
44	314.9	29.2	.66	44.5	22.2	.50
60	305.5	38.6	.64	54.8	30.0	.50
80	304.7	39.4	.49	74.5[6]	44.7[6]	.51
100	276.7	67.4	.67	82.3	50.6	.51
123	264.9	79.2	.64	98.0	62.4	.51
151	242.5	101.6	.67	116.9	76.6	.51
185	229.6	114.5	.62	138.8	93.1	.50
245	192.5	151.6	.62	178.3	122.7	.50
323	164.2	179.9	.56	224.0	156.9	.48
420	122.1	222.0	.53	275.2	195.3	.46
549	85.6	258.5	.47	321.7	230.1	.42
702	69.6	274.5	.39	339.8	243.6	.35

is soluble in 73 percent alcohol, and the amount of this material increases with the time of digestion.

Calculating the digestion per unit of time, it would seem that, measured by either method, the amount of starch digested is proportional to the time for the first 245 minutes or until about 44 percent of the starch is in such form as is soluble in 73 percent alcohol. From that point on, the starch digested per unit of time steadily falls off.

The close correspondence of the two methods can be noted from the curves in figures 2 and 3, in which the time in minutes is given on the abscissæ and the amount of digestion in milligrams on the ordinates. In figure 2 the line parallel to the base at 344 mg. represents the starch content of the original solution per 20 cc. as determined by alcoholic precipitation. By acid hydrolysis and sugar determination the starch content of the original solution was found to be 367.6 mg. per 20 cc. In figure 2 the results are plotted for the entire 702 minutes, in figure 3 for the first 151 minutes only. The latter figure shows clearly that, under the conditions of the experiment, in the earlier stages of diastatic action the amount of starch digested, measured by either method, is proportional to the time.

It seemed probable that the difference between the digestion, as determined by the maltose produced and by the material precipitated

[6] Time for this determination was 87.5 min.

in acid alcohol, could be largely overcome if a concentration of alcohol stronger than 73 percent were used as the precipitating agent. The influence of time on diastatic action was therefore determined by precipitating the starch and dextrins in 86 percent, in 73 percent, and in 60 percent acid alcohol. As was expected, the stronger the alcohol the greater was the precipitate. The close parallelism, however, between the curve for the precipitates in 86 percent alcohol and the

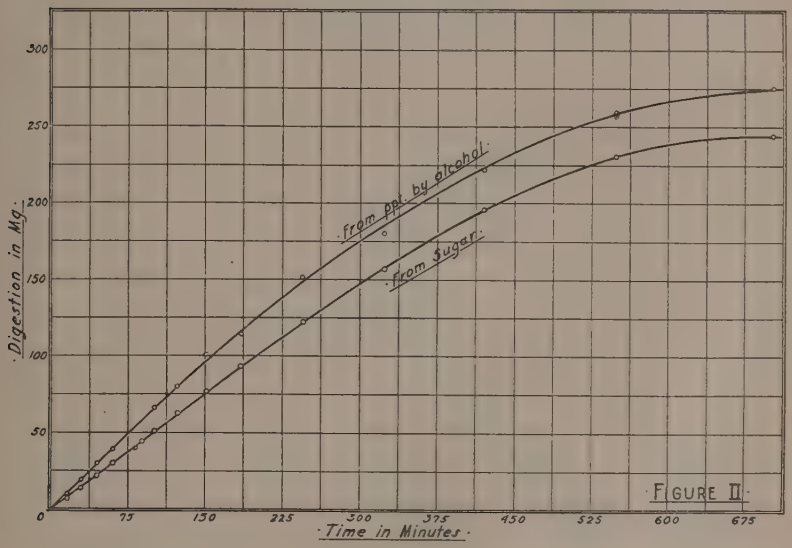

FIG. 2.

curve for the precipitates in 73 percent alcohol seemed to justify the use of the weaker strength. It is advantageous to use 73 percent alcohol (70 cc. of 95 percent alcohol + 1 cc. of HCl + 20 cc. of the solution) because the smaller quantity of liquid facilitates filtration and other mechanical operations incident to the determination.

In all the determinations the alcohol was acidified with HCl in order to facilitate the flocculation of the starch and to prevent the precipitation of the salts present. It was found by experiment that the concentration of the HCl used is more or less a matter of indifference. The amount of precipitate at various stages of digestion in

alcohol acidified with 1 cc. of HCl (sp. gr. 1.18–1.19), and in alcohol acidified with 1 cc. of acid one third of this strength, were identical.

From a consideration of the preceding data it would seem that the precipitation of starch and dextrins in 73 percent acid alcohol is a method that can be used with considerable satisfaction for the determination of diastatic action.

Preparation of Cultures and Use of the Alcoholic Precipitation Method for Determining Digestion.—The culture solutions in which single salts were used were prepared as follows: One liter of a solution

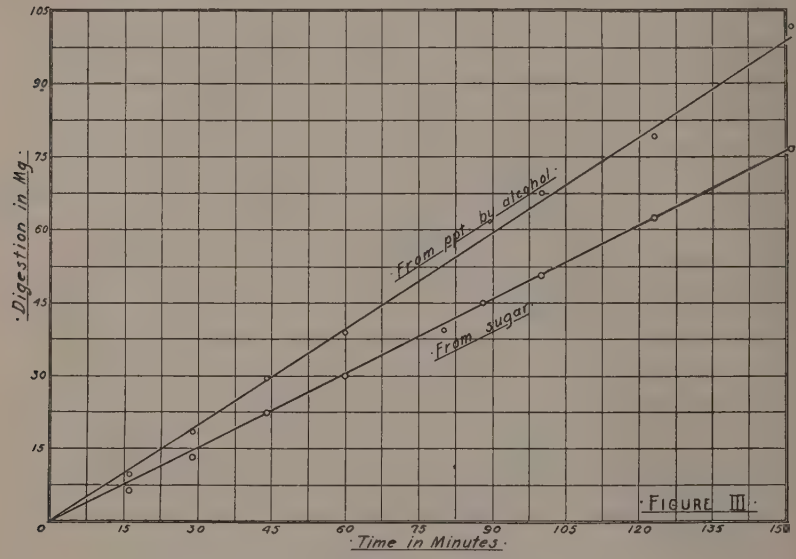

FIG. 3.

of M/100 concentration of the salt to be tested was prepared, and a stock solution of 1.6 percent soluble starch was also prepared by heating in an autoclav at a pressure of 15 pounds. The various concentrations of the salt—M/1000, M/10,000, etc.—were made by diluting the original salt solution and mixing with the starch solution so that the starch content of each series was approximately constant. Each culture contained 50 cc. of solution in an Erlenmeyer flask of 125 cc. capacity. The cultures were sterilized in an autoclav at 15

pounds pressure for 15 minutes. The culture solutions and vessels were weighed before sterilization, and just before analyzing the solution, each flask with its contents was brought up to the original weight by the addition of water. This was necessary in order to correct the change in concentration of the solution which results from the evaporation of water through the cotton plug of the flask.

The analysis of the original starch content was made after sterilization. After growth had occurred, the fungous mycelium was removed by filtering into a Gooch crucible to determine its dry weight after the method of Knudson (1913). The filtrate was caught in a 100 cc. test tube. Twenty cubic centimeters of this filtrate were removed and the undigested starch was determined by the alcoholic precipitation method described. The acidity or alkalinity of the filtrate to phenolphthalein and methyl orange was noted. All determinations were made in triplicate except the determinations of the original starch content, which in most cases was made in duplicate.

Method of Inoculating Cultures.—In inoculation the spores from the stock culture of the fungus were transferred by means of a sterile platinum needle to a second test tube containing sterile redistilled water. An equal number of drops of this spore suspension was added by means of a sterile pipette to each culture flask. This method of inoculation removes the danger of transferring soluble and suspended matter derived from the original stock culture to the experimental culture medium, as occurs in the method described by Hasselbring (1908). This modification was found necessary in view of the extreme sensitiveness of the fungus used to traces of inorganic nutrients, and of the possibility of introducing fragments of mycelium and organic matter. It was found that the same number of drops of the spore suspension must be used for each flask in a given series, because, within limits, the number of spores used in inoculation influences the amount of digestion. This is shown by the following experiment:

A set of 24 cultures was prepared, containing 0.8 percent of starch in distilled water which had previously been shaken up with cane sugar charcoal. One half of these cultures was inoculated with 3 drops of a suspension of spores of *Penicillium camembertii* in sterile distilled water, while to the other half 21 drops of the suspension were added. The results summarized in Table III are the average of triplicate cultures:

TABLE III

Soluble Starch in	Duration in Days	Original Starch Content (Mg. per 20 Cc.)	Starch Content After Digestion (Mg. per 20 Cc.)	Starch Digested (Mg. per 20 Cc.)	Dry Weight of Mycelium (Mg.)	Mg. of Starch Digested per Mg. of Dry Weight
3 drops of inoculum	7	141.9±.7	112.0±1.3	29.9	2.5±.1	11.9
21 drops of inoculum	7	141.9±.7	93.6± .9	48.3	2.3±.1	21.0
3 drops of inoculum	14	141.9±.7	79.7± .8	62.2	3.5±.1	17.7
21 drops of inoculum	14	141.9±.7	51.3± .8	90.6	3.1±.1	29.2

It is evident that any great difference in the number of spores used in inoculating the medium will produce considerable difference in the digestion, even though it may produce no effect on the detectable amount of mycelium formed.

INFLUENCE OF INORGANIC ELEMENTS ON THE SECRETION OF ENZYMES

Historical

Though considerable work has been reported by Katz, Went, Euler, Dox, Knudson, Kylin, and others on the relation of organic substances to the production and secretion of enzymes, relatively little has been reported on the relation of inorganic substances to the production and secretion of enzymes.

Fernbach (1890) concluded that the invertase formation by yeasts was influenced more by the source of nitrogen than by the source of carbon.

Effront (1902) states that phosphates, which influence yeast very favorably, are, on the contrary, unfavorable to the formation of invertase.

Saito (1910) investigated the formation of diastase by *Aspergillus Oryzæ* when grown in nutrient cultures containing either glucose, fructose, sucrose, maltose, galactose, lactose, or glycerol as sources of carbon, and either Witte's peptone, tyrosine, leucine, alanine, glycerol, asparagin, urea, ammonium tartrate, ammonium oxalate, ammonium chloride, ammonium sulfate, ammonium nitrate, dihydrogen ammonium phosphate, potassium nitrate, or calcium nitrate as sources of nitrogen. He tested the nutrient medium for diastase, and if it was lacking there he examined the mycelium for its presence. With nitrogen supplied to the nutrient solution in organic combination, diastase was always produced. With ammonium sulfate or ammonium chloride as the source of nitrogen, diastase was

not formed, save when starch was the source of carbon, in which case the enzyme was found only in the mycelium. Saito concluded that the source of nitrogen is significant in the formation of diastase.

Stoward (1911) grew barley embryos for from four to eight days in gelatine, and found that a mixture of asparagin and mineral salts, consisting of $CaSO_4$, KCl, $MgSO_4$, KH_2PO_4, and $FeCl_3$, influences the secretion of diastase more favorably than either the asparagin or the mineral salts alone. After determining the effect of the mineral salts on the activity of the secreted diastase, he concludes that they enter in some way into the metabolism of the embryo and thereby influence its secretory function.

Javillier (1912) noted that *Aspergillus niger*, grown in Raulin's solution lacking zinc, secreted invertase sufficiently rapidly to invert saccharose, but that the quantity secreted, calculated per unit of dry weight of the fungus, was noticeably less than was produced by the fungus in a complete solution.

Euler and Meyer (1912) suspended yeast cells in solutions of various substances for times varying from 20 to 150 hours, filtered off the yeast, and determined the inverting power of a unit weight of this living yeast. They found that by suspending the yeast in a solution of 4 g. of asparagin, glycocoll, or ammonium sulphate in 500 cc. of Lintner's solution, the effect on the formation of the enzyme invertase was beneficial and the same in each case.

Euler and Cramer (1913), working with the same problem, state that invertase formation is clearly bound up with the new formation of protoplasm. The building up of protoplasm, however, is dependent on, first, the fermentation that supplies energy, and second, the presence of suitable nitrogenous material in the medium. When the solution used in the treatment of the yeast contains no nitrogenous material, invertase formation occurs, but in slight amount.

It is evident that information on the problem of the influence of inorganic salts on enzyme secretion is extremely meager.

The Effect of Single Salts on the Digestion of Starch by Penicillium camembertii

The effect of the chlorides, the sulphates, the dihydrogen phosphates, and the nitrates of sodium and potassium, and of the chlorides. the sulphates, and the nitrates of calcium and magnesium, when

present singly in solution, on the growth of *Penicillium camembertii* and on the digestion of starch by that fungus, was determined after the methods given above.

KCl and NaCl.—In this experiment various concentrations of KCl or NaCl were used. The fungus was grown in the dark at 25° C., and the amount of starch digested was determined at the end of one and of two weeks. The results summarized in Table IV are the averages for triplicate cultures. Where no probable error is given for these values, it is less than 0.1 mg.

TABLE IV

KCl and NaCl

Soluble Starch in	Duration in Days	Original Starch Content (Mg. per 20 Cc.)	Starch Content after Digestion (Mg. per 20 Cc.)	Starch Digested (Mg. per 20 Cc.)	Dry Weight of Mycelium	Mg. of Starch Digested per Mg. of Dry Weight
M/1000 KCl.....	7	134.1± .8	103.8± .4	30.3± .9	1.5±.1	20.2±1.4
3M/10,000 KCl...	7	133.1± .9	99.5±1.3	33.6±1.6	1.4±.1	24.0±2.0
M/100,000 KCl...	7	133.5± .1	101.2±1.4	32.3±1.4	1.5±.1	21.5±1.6
Water treated with carbon black....	7	145.6±1.3	101.7±1.6	43.9±2.0	1.5±.1	29.2±2.3
M/100,000 NaCl..	7	143.8±1.0	103.4± .6	40.4±1.1	1.3	31.0±2.5
M/10,000 NaCl...	7	140.8± .1	104.1±3.0	36.7±3.0	1.5±.1	24.4±3.8
M/1000 NaCl.....	7	142.9	103.7± .9	39.2± .9	1.5±.1	26.1±1.8
M/1000 KCl.....	14	134.1± .8	67.3± .7	66.8±1.1	2.0	33.4±1.8
3M/10,000 KCl...	14	133.1± .9	73.3± .6	59.8±1.1	1.8	33.2±2.0
M/100,000 KCl...	14	133.5± .1	71.1± .5	62.4± .5	1.7±.1	37.0±2.2
Water treated with carbon black....	14	145.6±1.3	58.5±1.	87.1±1.6	2.2±.1	39.6±1.9
M/100,000 NaCl..	14	143.8±1.0	67.4±1.5	76.4±1.8	2.2±.1	34.7±1.8
M/10,000 NaCl...	14	140.8± .1	69.2±1.8	71.6±1.8	2.1±.1	34.1±1.5
M/1000 NaCl.....	14	142.9	73.5±2.3	69.4±2.3	2.2±.1	35.1±1.8

In Table IV the probable errors have been calculated for the "starch digested," which is the difference of the two quantities "original starch content" and "starch content after digestion." They have also been calculated for the "mg. of starch digested per mg. of dry weight," which is the quotient of the starch digested in a culture divided by the dry weight of the mycelium produced in the same culture. In all later tables this calculation has not been made, though the probable errors are given for the averages of the determinations made on the original starch content, the starch content after digestion, and the dry weight of the mycelium.

From the data given in Table IV, it can be observed that, in general, KCl and NaCl decrease the amount of starch digested by *Penicillium camembertii*. This inhibition is evident in the case of both KCl and

NaCl at a concentration of M/100,000. The amount of salt producing an evident effect is very small. In the case of KCl, 50 cc. (the volume of each culture solution) of a M/100,000 solution contains about .000037 g. of KCl and the same concentration of NaCl contains a little less than .00003 g. That the effect of such a small amount of a neutral salt on the digestion of starch by a fungus could be measured might be doubted, if the results obtained at the end of the first week were not substantiated by the results obtained at the end of the second week.

It can also be observed that with increasing concentration of the salts the amount of starch digested is decreased, with three exceptions —M/100,000 KCl and M/1000 NaCl at the end of the first week, and M/1000 KCl at the end of the second week. None of these aberrant results, however, are verified in both determinations. This inhibiting effect of the salts on digestion is also evident when the loss per unit weight of fungus is considered, with the exceptions already referred to.

It may also be noted that in every case the absolute amount of starch digested is less in the presence of KCl than in the corresponding concentration of NaCl. The same is true with respect to the amount of starch digested per unit of dry weight of mycelium. Two exceptions to this last statement may be noted, namely, the M/1000 and M/100,000 concentrations at the end of the second week.

TABLE V

K_2SO_4 and Na_2SO_4

Soluble Starch in	Duration in Days	Original Starch Content (Mg. per 20 Cc.)	Starch Content After Digestion (Mg. per 20 Cc.)	Starch Digested (Mg. per 20 Cc.)	Dry Weight Mycelium (Mg.)	Mg. of Starch Digested per Mg of Dry Weight
M/1000 K_2SO_4.....	7	137.2± .9	113.7±1.1	23.5	3.2±.1	7.3
M/10,000 K_2SO_4.....	7	136.3± .5	115.3	21.0	3.1±.1	6.8
M/100,000 K_2SO_4....	7	139.8±1.1	117.6±3.2	22.2	3.0±.1	7.4
Water treated with carbon black.....	7	137.8±1.3	111.5± .5	26.3	3.0±.1	8.8
M/100,000 Na_2SO_4...	7	139.7±1.2	114.9± .5	24.8	3.3±.1	7.5
M/10,000 Na_2SO_4....	7	137.1± .3	109. ±1.1	28.1	3.3±.1	8.5
M/1000 Na_2SO_4.....	7	135.9± .3	108.3±1.5	27.6	3.4±.1	8.1
M/1000 K_2SO_4.....	15	137.2± .9	78.5±1.2	58.7	4.0	14.7
M/10,000 K_2SO_4.....	15	136.3± .4	88.2	48.1	4.1	11.7
M/100,000 K_2SO_4....	15	139.8±1.1	75.6± .6	64.2	4.2±.2	15.3
Water treated with carbon black.....	15	137.8±1.3	64.5± .8	73.3	4.0	18.3
M/100,000 Na_2SO_4...	15	139.7±1.2	76.0±1.3	63.7	4.3±.1	14.8
M/10,000 Na_2SO_4....	15	137.1± .3	76.4±2.4	60.7	3.9±.1	15.6
M/1000 Na_2SO_4.....	15	135.9± .3	70.1± .9	65.8	4.0	16.4

K_2SO_4 and Na_2SO_4.—In this series *Penicillium camembertii* was grown in various concentrations of K_2SO_4 or Na_2SO_4. Incubation of cultures was made in the dark at 25° C. The data summarized in Table V are the averages of triplicate cultures.

From the data in Table V it may be noted that with K_2SO_4 and Na_2SO_4 much the same effect results as with the chlorides of the same bases. An inhibition in the actual amount of starch digested is produced even by a concentration of M/100,000. The effect of increasing concentration of the salts on the digestion is not so evident as with the chlorides. The M/1000 concentrations are clear exceptions, the digestion being increased over M/10,000 in both cases and in both weeks. This would appear to be a real effect, not one due to errors in determination. It can also be observed in this case that the K_2SO_4 has a greater inhibitory effect on digestion than the Na_2SO_4. This is shown in every case but one, the M/100,000 concentrations at the end of two weeks, in which the difference is very slight.

KNO_3 and $NaNO_3$.—Penicillium camembertii was grown in this case in the concentrations of KNO_3 or $NaNO_3$ noted in Table VI. The cultural conditions were those already noted. The results given in Table VI are the averages of triplicate cultures:

TABLE VI

KNO_3 and $NaNO_3$

Soluble Starch in	Duration in Days	Original Starch Content (Mg. per 20 Cc.)	Starch Content After Digestion (Mg. per 20 Cc.)	Starch Digested (Mg. per 20 Cc.)	Dry Weight of Mycelium (Mg.)	Mg. of Starch Digested per Mg. of Dry Weight
M/1000 KNO_3.....	7	140.9± .9	12.9±3.1	128.0	15.7±1.7	8.1
M/10,000 KNO_3....	7	137.4± .1	53.8±3.6	83.6	9.4± .3	8.9
M/100,000 KNO_3...	7	138.7± .1	74.1±2.2	64.6	3.7± .1	17.5
Water treated with carbon black.....	7	143.1±1.1	68.4± .5	74.7	3.7± .1	20.2
M/100,000 $NaNO_3$..	7	136.4± .1	66.1±1.4	70.3	4.0± .1	17.6
M/10,000 $NaNO_3$...	7	137.8± .8	47.7±1.3	90.1	7.6± .3	11.8
M/1000 $NaNO_3$.....	7	131.8± .1	13.1± .8	118.7	7.2± .1	16.5
M/1000 KNO_3.....	14	140.9± .9	Complete digestion		32.1± .4	——
M/10,000 KNO_3....	14	137.4± .1	10.7±1.5	126.7	12.8± .1	9.9
M/100,000 KNO_3...	14	138.7± .1	36.1± .8	102.6	5.3	19.3
Water treated with carbon black.....	14	143.1±1.1	34.3± .6	108.8	4.7	23.1
M/100,000 $NaNO_3$..	14	136.4± .1	24.9±2.0	111.5	5.3± .1	21.0
M/10,000 $NaNO_3$...	14	137.8± .8	9.0± .8	128.8	10.0± .2	12.9
M/1000 $NaNO_3$.....	14	131.8± .1	Complete digestion		13.5± .2	——

With the nitrates of potassium and sodium (Table VI) there is an increased growth which is greater in the KNO_3 than in the $NaNO_3$. We see, however, that with one exception—that of M/100,000 $NaNO_3$ at the end of two weeks—the M/100,000 concentrations decrease the actual amount of digestion, even though the amount of growth is increased in those concentrations. If the digestion per unit weight of fungus is considered, it is evident that the digestion decreases with increasing amounts of salt.

It can also be observed here that with the exception of the M/1000 concentration, which might be accounted for by the great difference in growth in favor of the KNO_3 culture, there is less digestion than in the corresponding strength of $NaNO_3$. This is also evident in the digestion per unit weight of mycelium, in which the digestion per unit of dry weight in the M/1000 concentration of the two salts agrees with the general proposition.

KH_2PO_4 and NaH_2PO_4.—In this series the fungus was grown in the presence of various concentrations of KH_2PO_4 or NaH_2PO_4. The cultural conditions were as before noted. The results summarized in Table VII are the averages of triplicate cultures:

TABLE VII

KH_2PO_4 and NaH_2PO_4

Soluble Starch in	Duration in Days	Original Starch Content (Mg. per 20 Cc.)	Starch Content after Digestion (Mg. per 20 Cc.)	Starch Digested (Mg. per 20 Cc.)	Dry Weight of Mycelium (Mg.)	Mg. of Starch Digested per Mg. of Dry Weight
M/1000 KH_2PO_4.....	7	137.1± .1	102.8±2.7	24.3	2.3±.1	10.5
M/10,000 KH_2PO_4.....	7	140.3± .2	107.9±2.2	32.4	2.7±.1	12.0
M/100,000 KH_2PO_4.....	7	139.7±1.6	104.7±1.1	35.0	2.2±.1	15.9
Water treated with carbon black.....	7	134.3± .6	100.8±1.	33.5	2.0±.1	16.7
M/100,000 NaH_2PO_4....	7	140.9± .2	110.1±2.2	30.8	2.2±.1	14.0
M/10,000 NaH_2PO_4.....	7	139.8	101.8± .8	38.0	2.3±.1	16.5
M/1000 NaH_2PO_4.....	7	140.4	106.3± .5	34.1	2.4±.2	14.2
M/1000 KH_2PO_4.....	14	137.1± .1	80.7±2.1	56.4	3.3	17.1
M/10,000 KH_2PO_4.....	14	140.3± .2	69.6± .5	70.7	3.1±.1	22.8
M/100,000 KH_2PO_4.....	14	139.7±1.6	74.1± .8	65.6	3.3±.1	19.9
Water treated with carbon black.....	14	134.3± .6	67.6± .8	66.7	3.3±.3	20.2
M/100,000 NaH_2PO_4....	14	140.9± .2	73.9± .5	67.0	3.4±.1	19.7
M/10,000 NaH_2PO_4.....	14	139.8	70.6± .3	69.2	3.3±.1	21.0
M/1000 NaH_2PO_4.....	14	140.4	70.5±1.3	69.9	3.3	21.2

Analyzing the data given in Table VII, we note that M/1000 KH_2PO_4 is the only solution which produces marked decrease in the

amount of starch digested as compared to the water culture. In fact, from the loss noted in the NaH_2PO_4 series, NaH_2PO_4 appears to increase the amount of starch digested.

Again the potassium salt permits less digestion than the sodium salt, with two exceptions—M/100,000 concentrations at the end of the second week. It would appear that this failure to inhibit the digestion as compared to the check is due to the effect of the acid, not the basic, radical of these salts.

Ca and Mg salts.—Experiments similar to the above were performed, in which the chlorides, the sulphates, and the nitrates of calcium and magnesium were compared. Space precludes the citation of the actual data obtained. It will be sufficient to say that the results were very similar to those obtained with the potassium and sodium salts of the same acid radicals. The addition of M/100,000 of either $CaCl_2$, $MgCl_2$, $CaSO_4$, or $MgSO_4$ to distilled water treated with carbon black is sufficient to decrease the actual amount of digestion. The effect of this concentration of these salts on the growth of the fungus is inappreciable. $CaCl_2$, $MgCl_2$, $CaSO_4$, or $MgSO_4$ of a concentration M/10,000 depresses the actual digestion more than does a M/100,000 concentration of the same salts. The effect of this concentration on the dry weight of the fungus is also inappreciable. The amount of starch digested in M/1,000 concentration of these salts is greater than the digestion in M/10,000 concentration, but it is not so great as in the distilled water treated with carbon black. M/1,000 concentration of these four salts also increases the amount of growth. The dry weight of the fungous mycelium is from 0.5 to 1 mg. greater in the M/1,000 concentration than in the distilled water treated with carbon black or in the M/100,000 or M/10,000 concentration of these salts. The starch digestion per unit of dry weight is greatest in the distilled water treated with carbon black, and decreases with increasing concentration of the salt added. The addition of $Ca(NO_3)_2$ or $Mg(NO_3)_2$ to a solution of soluble starch in distilled water shaken up with carbon black increases the amount of digestion. It also increases the dry weight of the fungus. The digestion per unit of dry weight of mycelium, however, is less in the presence of the salt than in the water alone, and decreases with increasing concentration of the salt.

Discussion.—The results of this investigation give no support to the idea that potassium and calcium are intimately connected

with diastase formation. Neither would it appear that sulphur, chlorine, magnesium, and sodium are closely connected with diastase formation. The addition of traces of the salts containing these elements produces no increase in the rate of digestion of starch. It is recognized, of course, that an abnormal condition is presented for the growth of the fungus when only a single salt is supplied, because the absence of other salts must be a limiting factor in the use of the one supplied. Nevertheless, if one of the nutrients mentioned above were specifically concerned in diastase formation, it might be expected that increased digestion would occur in its presence. No such increase is noted.

On the contrary, it has been found that the sulphates and the chlorides of potassium, sodium, calcium and magnesium in M/10,000 and M/100,000 concentrations decrease the rate of digestion. The cause of this inhibition is obscure.

There seem to be two possibilities: either the decreased digestion is due to an inhibition of the activity of the secreted diastase by the salt, or the effect is physiological; one of decreased secretion.

From a consideration of recent work on the effect of salts on the activity of diastase, the writer is led to believe that the effect is physiological. Recent work seems to show that if the salts at the concentrations used here have any effect on the activity of diastase, it should be one of acceleration of action rather than retardation. Hawkins (1913), working with malt diastase, determined the effect of NaCl and KCl, in concentrations varying from 2M to M/2,048; of $CaCl_2$, in concentrations varying from 1M to M/4,096; and of $MgCl_2$, in concentrations varying from M/2 to M/8,192. NaCl and KCl produced a retardation—15 percent at M/128, and 5 percent and 7 percent, respectively, at M/512—yet they had no effect at a concentration of M/2,048, and in all higher concentrations they produced a marked acceleration. $CaCl_2$, in all the concentrations used, accelerated the action of diastase; and $MgCl_2$, in all concentrations save M/8,192, which had no effect, also accelerated the digestion.

VanLaer (1913) states that in a medium of amphoteric reaction (à reaction amphotére), alkaline to methyl orange and acid to phenolphthalein, small quantities of the neutral electrolytes are indispensable to the manifestation of the properties of diastase.

In every determination of the reaction of the medium in this investigation where single salts were used, it was found that it was

alkaline to methyl orange and acid to phenolphthalein. The determinations were made each time a culture solution was analyzed.

Sherman and Thomas (1915), working with a purified starch and a carefully prepared diastase, find an acceleration with NaCl, KCl, $NaNO_3$, Na_2SO_4, NaH_2PO_4, and KH_2PO_4 and state: "In our experiments it has been observed that as long as commercial starch, even of high grade, was used as a substrate, the smaller additions of the salts above mentioned have very little effect . . . ." It is therefore justifiable to assert that the chlorides and the sulphates of potassium, sodium, calcium and magnesium in M/100,000 and M/10,000 concentrations in distilled water treated with carbon black decrease the secretion of diastase. What the significance is of the fact that the potassium salts decrease the secretion more than do the sodium salts of the same acid radical, cannot be stated.

The fact that the addition of these salts to distilled water decreases the secretion of the enzyme diastase would seem to bear directly on the problem of the effect of distilled water on the growth of plants.

It is of interest to note in this connection that Merrill (1915) has recently found that boiling the distilled water in which the roots of seedlings have been immersed decreases its toxicity. The effect of the boiling has been ascribed by Merrill to a destruction of bacteria. It would seem possible that it might be due to the destruction of harmful enzymes or thermolabile toxines secreted in larger quantity in distilled water.

The conclusions reached by True (1914) on the leaching effect of distilled water on the roots of seedlings of *Lupinus albus* and the protective action of $CaCl_2$ on the growth of the roots also seem of particular interest. True concludes that in the presence of $CaCl_2$ the dissociating power of the distilled water over the proteids and other chemical mechanisms of the cell is largely undeveloped, and the chemical integrity of the cell is protected in some way unknown. Similarly it might be postulated that the salts used here prevent the separation of the enzyme from a union with the protoplasm. It should be stated, however, that True and Bartlett (1915) report that solutions of KH_2PO_4 and KCl act essentially like distilled water.

It has been noted that the phosphates of potassium and sodium do not inhibit the digestion, and that greatly increased digestion is obtained with the addition of the nitrates to solutions of starch in distilled water treated with carbon black. This might lend some

credence to the view that phosphorus and nitrogen are connected with diastase formation. The results secured with phosphorus are, however, hardly definite enough to allow one to draw conclusions very rigidly. The increased growth in the presence of nitrogen may also explain the increased secretion as the secretion per unit of dry weight of mycelium produced decreases with increasing concentration of the nitrates.

Digestion in Nutrient Solutions

The experiments on the influence of single salts revealed no evidence respecting the rôle of the elements in enzyme formation or secretion. It has already been stated that the absence of other salts might be limiting factors in the functioning of any given salt. Consequently, a series of experiments were performed to test the effect of the absence of various essential elements from the full nutrient solution.

In the first experiment a modification of Richards's (1897) medium[7] was employed.

The salts substituted were used in the same concentration as the originals and the substitutions made were as follows:

Minus nitrogen,	KNO_3,	replaced by	KCl
" potassium,	KNO_3,	"	"	$Ca(NO_3)_2$
"	KH_2PO_4,	"	"	$Ca(H_2PO_4)_2$
" phosphorus,	KH_2PO_4,	"	"	K_2SO_4
" magnesium,	$MgSO_4$,	"	"	Na_2SO_4
" sulphur,	$MgSO_4$,	"	"	$MgCl_2$
" iron,	$FeCl_3$,	"	"	NaCl

Two series of experiments were performed, in one of which 50 cc. of solution and 0.4 percent of starch were used, and in the other 500 cc. of solution and 0.8 percent of starch. Redistilled water was used as the solvent. The cultures were grown in triplicate in the dark at room temperature, and the time required for complete digestion of the starch was determined by daily tests of the culture medium by the method of Katz (1898).

[7] The medium used was composed of

KH_2PO_4	5 g.
$MgSO_4$	25 g.
KNO_3	1 g.
$FeCl_3$	Trace
Water	100 cc.
Starch	as indicated.

Though the dry weight of the mycelium was not determined (as each culture showed complete digestion), the amount of mycelium formed in all cultures, with the exception of that in the medium lacking iron, was noticeably less than in the full nutrient solution. The mycelium was least in the culture lacking all nutrients and in the culture lacking nitrogen. The growth in the culture lacking potassium differed in appearance from that in the other cultures in consisting of a large number of small bunches of mycelium, and not a continuous fluffy mass.

Certain differences were noted between the two sets of cultures in the relation of fruiting to digestion. All the cultures of the set containing 50 cc. of solution completed digestion before fruiting. In the set in which 500 cc. of solution was used, the full nutrient culture, and the cultures lacking phosphorus, sulphur, and iron, fruited before completing digestion, while the cultures lacking magnesium, nitrogen, and potassium did not. It will be noted from the results of these experiments, which are summarized in Table VIII, that at room temperature *Penicillium camembertii* digests 0.2 g. of starch in 50 cc. of modified Richards's solution in 9 days. A deficiency of iron, sulphur, magnesium, and phosphorus has little effect on the time required for digestion. A lack of nitrogen, on the one hand, and of all nutrients, on the other hand, has approximately the same effect, tripling the time as compared to that of the full nutrient culture in which .2 g. of starch in 50 cc. of solution is used, and increasing the time from sixteen to nineteen times when 4 g. of starch is contained in 500 cc. of solution.

TABLE VIII

Medium	Time for Digestion in Days	
	50 Cc. of Solution .4 Percent of Starch	500 Cc. of Solution .8 Percent of Starch
Starch only	17	171+
Full nutrient	6	9
Full nutrient minus nitrogen	15	143
Full nutrient minus potassium	117+	171+
Full nutrient minus phosphorus	8	10
Full nutrient minus magnesium	9	17
Full nutrient minus sulphur	8	18
Full nutrient minus iron	6	12

The digestion in the absence of potassium is exceedingly slow. It was believed at first that here was a relation between potassium

and diastase secretion. This, however, is not the case, as is shown by the following experiment, in which a different solution[8] was employed and the rate of digestion of starch was noted both in the presence and in the absence of potassium. The time required for the digestion of the starch in 50 cc. of the medium containing no K nor Na, and of the starch in 50 cc. of the same medium containing KH_2PO_4 or NaH_2PO_4 as indicated in Table IX, was determined by the method of Katz. Triplicate cultures were grown in the dark at room temperature, and the dry weight of the mycelium was determined as each culture showed complete disappearance of the starch.

TABLE IX

50 Cc. of Medium Plus	Time for Digestion (Days)	Dry Weight of Mycelium (Mg.)
M/10 KH_2PO_4	6	14.7
M/100 KH_2PO_4	6	11.1
M/1000 KH_2PO_4	4	6.4
M/10,000 KH_2PO_4	6	10.6
M/100,000 KH_2PO_4	7	6.6
No phosphate	8	5.1
M/10 NaH_2PO_4	9	9.6
M/100 NaH_2PO_4	8	5.4
M/10,000 NaH_2PO_4	6	6.7
M/10,000 NaH_2PO_4	7	6.4
.4 percent starch only	11	Weight not taken

It can be noted from the number of days required for complete digestion given in Table IX that a deficiency of potassium has little effect on the time required for digestion. In the medium used here it requires *Penicillium camembertii* but 8 days to digest 0.2 g. of starch in a deficiency of both potassium and phosphorus, which differs by but one or two days from the time necessary for complete digestion when both these elements are present in abundance. It would therefore appear that the long period of digestion in the minus potassium cultures of Table IX is due not to the lack of potassium, but to some other cause. This cause is apparently an inhibitive action of the

[8] The nutritive solution used was composed of

$Ca(NO_3)_2$	0.236 g. (M/100)
$MgSO_4$	0.0246 g. (M/1000)
$FeCl_3$	Trace
Starch	0.4 percent
Water	100 cc.
KH_2PO_4 or NaH_2PO_4	As indicated.

medium lacking potassium on the activity of the diastase. Determinations of the digestion of starch by Taka diastase in this medium show a very marked retardation apparently due to the concentrations of $Ca(H_2PO_4)_2$ and $MgSO_4$ present.

That the results in Table VIII do not necessarily indicate a connection between potassium and diastase secretion is also shown by the following experiment.

The time for digestion of a given quantity of starch in Czapek's medium,[9] with the carbon supplied as .4 percent of soluble starch and in media in which each of the elements generally accepted as essential for the fungi was replaced by some other element, was determined. The substitutions made were as follows:

Minus nitrogen,	$NaNO_3$,	replaced by	NaCl
" potassium,	K_2HPO_4,	"	"	$CaHPO_4$
	KCl,	"	"	$Ca(NO_3)_2$
" phosphorus,	K_2HPO_4,	"	"	K_2SO_4
" magnesium,	$MgSO_4$,	"	"	K_2SO_4
" sulphur,	$MgSO_4$,	"	"	$MgCl_2$
	$FeSO_4$,	"	"	$FeCl_2$
" iron,	$FeSO_4$,	"	"	NaCl

Penicillium camembertii, *Aspergillus Oryzæ* (Alhbury) Cohn, *Mucor Rouxii* (Calm) Wehmer, and a species of *Fusarium* of the subulatum type, were used to inoculate these cultures. They were grown at room temperature and tested daily for starch by the method of Katz. As the culture medium showed complete disappearance of the starch the dry weight of the mycelium was determined.

It would appear from the data as summarized in Table X, which represent the averages of triplicate cultures, that nitrogen is the only element whose absence makes any considerable difference in the time required for digestion.

It is also noteworthy that these four fungi may be separated into two groups: the *Fusarium* sp. and *Mucor Rouxii*, which digest starch

[9] The composition of this medium is

$NaNO_3$	.2 g.
K_2HPO_4	.1 g.
$MgSO_4$	.05 g.
$FeSO_4$	.001 g.
KCl	.05 g.
Water	100 cc.

TABLE X

Medium 60 Cc.	Fusarium sp.		Mucor Rouxii		Aspergillus Oryzae		Penicillium Camembertii	
	Time for Digestion (Days)	Dry Weight of Mycelium (Mg.)	Time for Digestion (Days)	Dry Weight of Mycelium (Mg.)	Time for Digestion (Days)	Dry Weight of Mycelium (Mg.)	Time for Digestion (Days)	Dry Weight of Mycelium (Mg.)
.4 percent starch	249+ blue	—[10]	249+ blue	—[10]	7	.4	23	1.4
−K	11	18.7	121	—[10]	7	8.0	6	3.1
−N	249+ brown	10.6	249+ purple	—[10]	19	13.6	159+	12.3
−Mg	9	37.2	74	—[10]	7	3.4	6	2.2
−P	11	41.9	42	34.1	5	3.4	6	5.3
−S	13	34.8	68	—[10]	10	20.4	6	16.8
−Fe	11	53.7	24	40.0	7	14.9	6	13.1
Full nutrient	10	45.4	23	44.6	7	15.6	6	14.4

very slowly in the absence of nitrogen; and *Aspergillus Oryzæ* and *Penicillium camembertii*, which digest starch fairly rapidly in the absence of all nutrients.

It may also be noted that there is little correlation between the time required for digestion and the dry weight of the fungous mycelium. In the case of *Penicillium camembertii*, the same time (6 days) is required to digest approximately .24 g. of starch in the cultures lacking potassium, magnesium, phosphorus, sulphur, and iron, and in the full nutrient, but the dry weight of the mycelium in these cultures varies from 2.2 mg. to 16.8 mg. Somewhat similar results are shown by the data obtained with the three other fungi.

Discussion.—An examination of the complete data obtained in the experiments with nutrient cultures shows that again we have no evidence to demonstrate that potassium is concerned with diastase formation. With the exception of those cultures in which nitrogen was lacking, there are no marked differences in the times required for the starch digestion. It is significant, as shown in Table X, that a longer period is required for starch digestion by the fungus when grown in the culture medium lacking nitrogen only, than in the medium lacking all nutrients. It was found that the combination of salts used in the minus nitrogen medium decreases the time required for Taka diastase to change a given quantity of starch to the point at which it no longer colors with iodine. The longer period of time required for digestion in the minus nitrogen culture medium is, there-

[10] Dry weight not determined.

fore, due to a decreased secretion of diastase. As was found with the single salts, the presence of salts decreases the secretion of diastase.

Summary

I. A method of determining diastatic action in solutions of soluble starch by the precipitation of the undigested starch and a part of the dextrins in acid alcohol is described.

II. The addition of the chlorides and the sulphates of potassium, sodium, calcium and magnesium, singly, to a solution of Merck's soluble starch in distilled water treated with carbon black, decreases the amount of starch digested by *Penicillium camembertii* when the salts are present in M/10,000 and M/1,000 concentrations.

III. The nitrates of potassium, sodium, calcium, and magnesium, when present singly in M/1,000, M/10,000, and, in the case of the nitrates of calcium and magnesium, in M/100,000 concentrations, in a solution of Merck's soluble starch in distilled water treated with carbon black, increase the amount of starch digested by *Penicillium camembertii*.

IV. The addition, singly, of the nitrates of potassium, sodium, calcium, and magnesium to a solution of Merck's soluble starch in distilled water treated with carbon black, decreases the amount of starch digested by *Penicillium camembertii* per unit of dry weight of mycelium when the salts are present in M/1,000, M/10,000 and M/100,000 concentrations.

V. The dihydrogen phosphates of sodium and potassium, with the exception of M/1,000 KH_2PO_4, do not decrease the digestion of starch when present in M/1,000, M/10,000 and M/100,000 concentrations.

VI. Potassium salts inhibit the digestion of Merck's soluble starch in distilled water treated with carbon black more than do sodium salts.

VII. A marked difference is noted between the speed with which *Aspergillus Oryzæ* and *Penicillium camembertii* digest soluble starch in the absence of all added nutrients, and the rate of digestion by *Mucor Rouxii* and *Fusarium* sp.

VIII. No evidence was found to connect potassium and calcium with diastase formation.

IX. Nitrogen may bear an intimate relation to the formation of diastase by *Penicillium camembertii*.

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